Sexual dimorphism in a broadcast spawning coral, *Porites lobata*

Ricardo Mijail Pedraza-Pohlenz  
CIBNOR: Centro de Investigaciones Biologicas del Noroeste SC  
https://orcid.org/0000-0002-2603-3316

Eduardo F. Balart  
CIBNOR: Centro de Investigaciones Biologicas del Noroeste SC

J. J. Adolfo Tortolero-Langarica  
ICMYL: Universidad Nacional Autonoma de Mexico Instituto de Ciencias del Mar y Limnologia

Alma Paola Rodríguez-Troncoso  
Universidad de Guadalajara - Centro Universitario de la Costa Puerto Vallarta

Michael E. Hellberg  
Louisiana State University

Orión Norzagaray-López  
Universidad Autonoma de Baja California Instituto de Investigaciones Oceanologicas

Rafael A. Cabral-Tena  
Centro de Investigación Científica y de Educación Superior de Ensenada: Centro de Investigacion Científica y de Educacion Superior de Ensenada

Amílcar Leví Cupul-Magaña  
Universidad de Guadalajara Centro Universitario de la Costa Sur

David A. Paz-García  
CIBNOR: Centro de Investigaciones Biologicas del Noroeste SC  
https://orcid.org/0000-0002-1228-5221

Research Article

**Keywords:** Reproduction, Morphometrics, Phenotypic modularity, Porites

**Posted Date:** May 6th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1376851/v1

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

**Version of Record:** A version of this preprint was published at Frontiers in Marine Science on January 10th, 2023. See the published version at https://doi.org/10.3389/fmars.2022.1068391.
Abstract

Sexual selection in gonochoristic corals should vary with the sex of the colony, which may lead to sexual dimorphism. Because coral colonies possess a complex morphological architecture composed of subunits (corallites), the degree of coordinated change among these subunits may be subject to divergent selection between sexes. This study tested for sexual dimorphism in the corallites of a reef-building coral Porites lobata, a free-spawner, and compared this with dimorphism of massive congeners, the brooder P. panamensis. Corallite area for male P. lobata was 17% larger than for conspecific females, perhaps due to enabling greater sperm release and fertilization success. Phenotypic modularity analysis (i.e., ability of subsets of traits to vary in a coordinated manner) showed that the integration of skeletal traits was similar between sexes in P. lobata but higher in males than females in P. panamensis. High integration may result in a more stable corallite morphology, while lower integration may permit a greater capacity for plasticity. Our results demonstrate corallite traits can be sexually dimorphic in free-spawning corals and suggest that between-sex differences in the degree of corallite integration may vary with reproductive mode.

Introduction

Sexual dimorphism results from phenotypic differences between sexes of the same species and is often evidenced by conspicuous traits (e.g., size of anatomical structures, coloration) that vary between species (Berns 2013). Sexual dimorphism arises due to differences in trait optima between the sexes that stem from differences in mating strategies and the energetic cost of gametes (Parker 1979). For example, if energy is not limited, then an increase in the size of females compared to males could increase fitness through the production of more eggs (Fairbairn et al. 2007). Sexual dimorphism is widespread among animals, but has been considered rare in colonial marine taxa (Levitan 2010). Recent studies on scleractinian corals, however, have found sexual dimorphism in size (Kramarsky-Winter and Loya 1998; Loya and Sakai 2008), calcification rates (Cabral-Tena et al. 2013; Tortolero-Lagarica et al. 2016, 2017; Mozqueda-Torres et al. 2018; Cruz-Ortega et al. 2020), skeletal isotopic signals (Cabral-Tena et al. 2016), and corallite morphologies (González-Espinosa et al. 2018).

Clonal marine invertebrates are modular organisms (Magwene 2001; Eble 2005; Esteve-Altava 2017) and all start with a basic unit that clones to form a colony (Notov 2015). Reef-building corals show a modular organization at different levels: colony, branch, and their most fundamental unit, a single tentacled polyp, and the underlying skeleton (corallite) that supports it (Lasker et al. 2003; Shaish et al. 2007). Morphological plasticity can be driven by variation at each level of modularity (Witzany 2014). Modularity is defined as the ability of subsets of traits (i.e., modules) to vary independently of other such subsets while remaining associated with other traits within the subset (Magwene 2001). Traits will thus have higher integration within modules than between modules (Klingenberg 2014). The integration of traits in modules has been observed at the colony level during phenotypic shifts in octocorals (Sánchez and Lasker 2003; Sánchez et al. 2007) and scleractinian corals (Paz-García et al. 2015). If, however, sexual
selection is strong and acts on corallites, then both corallite traits and their modularity may vary between sexes.

Scleractinian corals show a range of sexuality (gonochorism or hermaphroditism) and reproductive modes (brooding or spawning) (Harrison 2011). Free-spawning corals release their gametes into the water column, where fertilization and larval development occur. Brooders release only sperm, with fertilization and maturation of larvae taking place within the polyps of females (Oliver and Babcock 1992; Baird et al. 2009; Harrison 2011). Different reproductive modes could carry a different energetic cost depending on the sex of the colony, which could lead to different levels of sexual dimorphism in reef-building species. In this study, we tested for sexual dimorphism in the corallite features of *P. lobata*, a broadcast spawning reef-builder that has shown evidence of sexual dimorphism in their skeleton growth (Tortolero-Langarica et al. 2016; Table S1). We then compared the integration of traits of this species with a congeneric brooding species (*P. panamensis*) in the context of sexual dimorphism.

### Material And Methods

#### Sample collection and sex identification

*Porites lobata* is one of the most abundant massive coral species along the Eastern Tropical Pacific (Glynn et al. 2017). We collected samples at Isla Isabel National Park in the central Mexican Pacific (Fig. 1) in October 2013 (permission number: DGOPA.04552.040711.1798). All colonies were collected from the same habitat (see, Tortolero-Langarica et al. 2016) within 3–5 m of each other to avoid phenotypic differences caused by different light or flow microenvironments. *P. lobata* can reproduce throughout the year in Panama (Glynn et al. 2011), although its reproductive activity has not been documented in Mexico. Specimens of *P. lobata* were morphologically identified as such when collected, although in the eastern tropical Pacific they can be difficult to distinguish from *Porites evermanni* (Boulay et al. 2014; Hellberg et al. 2016).

Colony sex was determined using the histological procedure of Cabral-Tena et al. (2013) and Tortolero-Langarica et al. (2016). Briefly, two fragments were collected from each colony. One (~ 5 x 5 cm) was decalcified to obtain tissue, dehydrated, and stained using Masson's trichrome protocol. Each colony was sexed by inspecting for the presence of oocytes or spermaries using a Zeiss AxioVision R1 microscope. The second fragment (~ 10 x 10 cm) was used for morphological analysis of the skeleton. Tissue was removed with 5% sodium hypochlorite to characterize their morphological traits.

### Morphological traits and analysis of modularity

#### Morphological traits and comparison between sexes

Five morphological traits were selected for analysis (Table 1) based on their potential to reveal sexual dimorphism in a congeneric species, *P. panamensis* (González-Espinosa et al. 2018). A Nikon D5300 digital camera was used to capture images for corallite density (Fig. 2a). Due to the small size of
corallites of *P. lobata* (Fig. 3c), high-resolution images were also obtained using a Hitachi S-3000N Scanning Electron Microscope (SEM). Those SEM images were used to measure morphological traits in 10 corallites per colony (Fig. 2b, Table 1). Morphological traits were analyzed using ImageJ ver. 1.60 software (https://imagej.nih.gov/ij/) (Schindelin et al. 2015). Images were calibrated with a grid with standard dimensions. Each trait measurement was then averaged to obtain a single mean value per colony. Statistical analyses were performed using colony means. After testing the data for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene test), the Student’s t-test was used to determine trait differences between sexes in *P. lobata*.

<table>
<thead>
<tr>
<th>Morphological trait</th>
<th>Abb.</th>
<th>Description</th>
<th>Sampling per colony</th>
<th>Unit</th>
<th>Measurement method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Corallite density</td>
<td>CDe</td>
<td>Total number of corallites per cm².</td>
<td>3–6 squares of 1 cm²</td>
<td>Count</td>
<td>Fragment image</td>
<td>1</td>
</tr>
<tr>
<td>2. Corallite diameter</td>
<td>CDi</td>
<td>Mean value between the linear distance from the initial-point of the dorsal septum to the initial-point of the ventral septum, and from the initial-point of the lateral septum to the end of the other lateral septum.</td>
<td>10 corallites (20 measurements)</td>
<td>mm</td>
<td>SEM image</td>
<td>1</td>
</tr>
<tr>
<td>3. Adjacent corallites</td>
<td>AC</td>
<td>Count of the total number of adjacent corallites.</td>
<td>10 central corallites</td>
<td>Count</td>
<td>SEM image</td>
<td>1</td>
</tr>
<tr>
<td>4. Distance of adjacent corallites</td>
<td>DaC</td>
<td>Average of the lineal distance from the centre of the corallite to the centre of the closest and farthest adjacent corallite.</td>
<td>10 corallites (20 measurements)</td>
<td>mm</td>
<td>SEM image</td>
<td>1</td>
</tr>
<tr>
<td>5. Corallite Area</td>
<td>CAr</td>
<td>Area of the whole corallite.</td>
<td>10 corallites</td>
<td>mm²</td>
<td>SEM image</td>
<td>1</td>
</tr>
</tbody>
</table>

We performed a canonical discriminant analysis (CDA) comparing sexual dimorphism observed in *P. lobata* (this study) with data for *P. panamensis* (https://doi.org/10.5061/dryad.5d6sp) from González-Espinosa et al. (2018). We performed a random partition of 50% of the total colonies of *P. panamensis* from the dataset to perform analysis with a similar sample size in both *Porites* species. We used four out of five morphological traits (corallite density, corallite diameter, distance of adjacent corallites, and number of adjacent corallites; Table 1) to perform the CDA. Corallite area was not used because it was not measured by González-Espinosa et al. (2018). All statistical analyses were conducted with STATISTICA 7 software (www.statsoft.com).

**Analysis of Modularity**

The four morphological traits used in the CDA (Table 1: traits 1–4) were also used for the modularity analysis. Integration between morphological traits was analyzed using conditional independence tests and independence graphs (Magwene 2001), which show the interaction between two traits after removing the effect of other traits and provide insight into whether traits are evolving in a coordinated manner or independently. Due to the number of traits measured in each species, second-order partial correlation coefficients (PCC) for both *Porites* species were computed from log-transformed data. The PCC was estimated for each set of two variables, controlling for the remaining variables (two). The PCC analyses were performed using STATISTICA 7.

Weak trait interactions were removed from the model by statistically comparing the PCCs for edge exclusion deviance (Magwene 2001). The strength of the interactions was calculated by the edge strength (Magwene 2001; Paz-García et al. 2015). For this model, we used four categories to classify the strength of the interactions based on the edge strengths results: weak integration (0-0.2), regular integration (0.21–0.4), strong integration (0.41–0.6), and very strong (> 0.6) integration between traits. A graphic model was constructed for each species and sex (2 species × 2 sex), resulting in 4 different models in total. The resulting graphical model represents morphological traits in nodes and integration among traits by lines (Fig. 5). The edge strength was represented in the graphical model as followed: weak integration (thin dotted line), regular integration (thick dotted line), strong integration (thin continuous line), and very strong integration (thick continuous line). The modularity level in each graphical model will depend on the strength (line thickness) and the number of trait connections (lines) for each model.

**Results**

**Sexual dimorphism in the free-spawning *Porites lobata***

We analyzed 49 colonies for the CDA and modularity analysis: 18 from *Porites lobata* (12 males, 6 females) and 31 from *Porites panamensis* (12 males, 19 females). In free-spawning species, *P. lobata*, morphological traits were larger in male colonies than female colonies (Fig. 3, Table S2). Sexual
dimorphism was statistically supported in the analysis of morphological traits (Fig. 3, Table S2), CDA (Fig. 4), and modularity analysis (Fig. 5). In *P. panamensis*, CDA (Fig. 4) and the modularity analysis (Fig. 5) likewise supported sexual dimorphism, as reported previously (González-Espinosa et al. 2018).

Sexual dimorphism in *P. lobata* was supported by two of five morphological traits analyzed (Table 1, Fig. 3). Male colonies had larger corallite areas (by 17%) and diameters (by 10%) than females (p < 0.05, Fig. 3, Table S2). For the CDA, the first axis explained 86.12% of the variance and the second axis explained an additional 10.41% between the species and sexes (Fig. 4). In both *P. lobata* and *P. panamensis*, there were differences between sexes (Wilk’s λ = 0.11596, approx. $F_{(12,111)} = 11.676$, p < 0.0001, Fig. 4). The CDA resulted in a correct classification rate above 66% with an average of 77% for males and females from both species (Table S3).

**Modularity differs between sexes**

Patterns of modularity differed between sexes for both species (Fig. 5, Tables S4-S5). The distance between adjacent corallites (DaC) showed the highest level of integration between traits in all four models (Fig. 5), while corallite density (Cde) also showed higher coordination between traits in both *Porites* species (Fig. 5). Female colonies showed higher integration than male colonies in *P. lobata*, while *P. panamensis* showed the opposite pattern.

For *P. lobata*, while the number of integrated traits was similar between sexes (two integrated traits for males, three for females), the connections between traits were different (Fig. 5a-b, Table S4). Corallite density was integrated with adjacent corallites (AC) in both sexes, but the other associations varied between the sexes. Corallite density, the distance of adjacent corallites, and the number of adjacent corallites were the most integrated traits in female colonies, showing two connections (Fig. 5b). In contrast, male colonies showed a unique connection between corallite diameter (CDi) and the distance of adjacent corallites (Fig. 5a).

In *P. panamensis*, males showed a higher integration of traits (4 connections between traits; Fig. 5c, Table S5) than females (1 connection between traits; Fig. 5d, Table S5). Corallite density and distance between adjacent corallites were the most integrated traits in both sexes (Fig. 5c-d).

**Discussion**

**Sexual dimorphism in coral reef-building species**

We found sexual dimorphism in the free-spawning coral *Porites lobata* in both corallite morphology and their level of integration among corallite traits. Evidence of sexual dimorphism in scleractinians has been found to differences in size within solitary corals (see Table S1) (Kramarsky-Winter and Loya 1998; Loya and Sakai 2008), skeletal growth, and calcification rates (see Table S1), as well as corallite morphology (González-Espinosa et al. 2018). In two gonochoric free-spawners from the family Fungiidae, *Fungia*
scutaria in the Red Sea (Kramarsky-Winter and Loya 1998) and Ctenactis echinate in the East China Sea (Loya and Sakai 2008), males are small whereas large individuals are all females. In massive Caribbean species, such as Montastraea cavernosa (Mozqueda-Torres et al. 2018), Dichocoenia stokesi, and Dendrogyra cylindrus (Cruz-Ortega et al. 2020), male colonies exhibit higher skeletal growth rates than female colonies. Likewise, in the eastern Pacific, P. lobata (Tortolero-Langarica et al. 2016), P. panamensis (Cabral-Tena et al. 2013; Tortolero-Langarica et al. 2017), and the hermaphrodite free-spawner Pavona gigantea (Tortolero-Langarica et al. 2017) show sexual dimorphism in annual skeletal growth, all with faster growth in male colonies.

Previous studies have suggested that female colonies may have less energy to invest in growth due to the high energetic cost of producing eggs (Hall and Hughes 1996; Cabral-Tena et al. 2013; Tortolero-Langarica et al. 2016, 2017). Males, in contrast, can invest more energy in growth due to the lower energetic cost of sperm production. These previous studies (Table S1), along with our new results on corallite morphology, suggest that sexual dimorphism in corals is common, regardless of phylogenetic lineage (seven genera), geographical location (Red Sea, East China Sea, Caribbean, and eastern Pacific) or spawning mode (this study), and that intersexual differences in corallite integration may facilitate plasticity that allows species to acclimate to different environmental conditions.

**Sexual dimorphism**

Sexual dimorphism tied to corallite size operates differently in free-spawner P. lobata (this study) than in brooding species P. panamensis (González-Espinosa et al. 2018). In free spawners, the corallite area was larger in male colonies than in female colonies (Fig. 3; Table S2). In contrast, P. panamensis showed larger corallites in female colonies compared to male colonies (González-Espinosa et al. 2018). Free-spawning females produce far smaller eggs than brooders, and there are no larvae to brood (Shlesinger et al. 1998; Glynn et al. 2017). Thus, suggesting a reduced selective pressure for female corallites (polyps) to increase their size, in contrast to male colonies, which should still face selective pressure to maximize reproductive success via increased sperm production (Levitan 2010).

Gamete production in different coral species is influenced by the number and size of gonad-bearing mesenteries in each polyp, which in turn are limited by the space available inside a polyp (Hall and Hughes 1996). As a result, a larger polyp (i.e., corallite area) could increase fecundity (Hall and Hughes 1996; Shlesinger et al. 1998; Leuzinger et al. 2003) by the production of larger testes, as in other free-spawning invertebrates (Evans and Sherman 2013). Both characteristics could potentially explain the larger corallite size in males of P. lobata (Fig. 3).

Corallite area has also been linked to trophic strategy, with variation in polyp size resulting from a trade-off between optimizing for autotrophy (small size) or heterotrophy (large size) (Conti-Jerpe et al. 2020). Porites species employ autotrophic and heterotrophic nutritional sources (Palardy et al. 2005; Grottoli et al. 2006). A larger corallite area in males could enhance the capture of particulate organic matter and
planktonic prey. Such heterotrophic nutrition sources lead to a higher tolerance to bleaching when the autotrophic benefits of symbiosis are disrupted (Conti-Jerpe et al. 2020).

The production of gametes is a coral most energetically costly physiological activity, especially for female colonies (Hall and Hughes 1996), sometimes diminishing other processes such as skeletal extension and calcification (Cabral-Tena et al. 2013; Tortolero-Lagarica et al. 2016, 2017). While reproductive output increases with polyp size, the ratio of reproductive output to somatic tissue decreases with a higher polyp diameter (Leuzinger et al. 2003). Larger polyps require thicker skeletal elements for support, thereby reducing the space available for reproductive tissue (Leuzinger et al. 2003). Based on this inverse relationship and the high energetic cost of gamete production, if there is no selective pressure for female corals to increase their corallite size (as in brooders), a reduced size in corallite size should be favored. Interestingly, the opposite trend has been observed in two solitary fungiids (gonochoric spawners), Fungia scutaria and Ctenactis echinate, where females have larger polyps than males (Kramarsky-Winter and Loya 1998; Loya and Sakai 2008). Sexual size dimorphism can vary depending on different selective pressures (e.g., environmental conditions), as seen in P. panamensis (González-Espinosa et al. 2018), and further studies should bring more insight into sexual dimorphism in scleractinian corals.

Integration of traits

The integration of traits in P. lobata was slightly higher for females than for males (Fig. 5a-b, Table S4). Integration may constrain the elements of a module (the corallite here) to evolve in concert due to directional selection on just one or a few traits in the module (Magwene 2006; Villmoare 2013; Klingenberg 2014). The overall integration between traits in P. lobata was much lower than in P. panamensis (Fig. 5, Tables S4-S5). Less integration, and thus weaker constraints, could allow for greater morphological plasticity to adjust to variable environmental conditions (Klingenberg 2014). Indeed, high morphological plasticity is characteristic of the corallites and whole colonies of P. lobata (Forsman et al. 2009). Intraspecific corallite variation is also high, showing differences between inshore and offshore colonies (Tisthammer and Richmond 2018).

In contrast, in the brooding P. panamensis trait integration was lower in females than in males (Fig. 5, Table S5). Each polyp in a female colony of P. panamensis can brood a single larva that can reach a diameter of 800 µm (Carpizo-Ituarte et al. 2011), potentially placing selective pressure to increase internal space within each corallite to harbor larger larvae (González-Espinosa et al. 2018). Strong selection for larger corallites in females could also alter the degree of integration of traits between colonies of different sexes and may allow female colonies independence of trait change and greater morphological plasticity. This plasticity could free them to vary their corallite structure depending on the environment (Todd 2008) to cope with energetic constraints. For example, this potential plasticity could include (1) increase in the size to accommodate larger larvae, (2) increase their energy supply by having a higher density of dinoflagellate algal symbionts, and (3) enable the polyps to feed on bigger particles.
Sexual dimorphism is supported by morphological and modularity analysis in *P. lobata*, while *P. panamensis* colonies showed differences between sexes in the integration between traits. Further studies should focus on species with distinct reproductive patterns (i.e., a hermaphroditic brooder/spawner) for further insight into the evolution of sexual dimorphism in scleractinian corals and should look to include more than five coral colonies per sex. Our findings also indicate that the corallite diameter and area can be used to identify the sex of *P. lobata* colonies and serve as a target characteristic in other corals to better understand their reproductive biology. The distinct patterns of sexual dimorphism observed between reproductive modes (larger females in brooders vs. larger males in spawners) may be related to the different selective pressures that each sex faces.

**Declarations**

**Ethics**

Permission to collect samples of *P. lobata* was granted by The Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food of Mexico (SAGARPA) (permit No. DGOPA.04552.040711.1798). The permitted collections were made in October 2013 at Isla Isabel National Park. Field collections were made by JATL, APRT, ALCM, and with the support of the authorities on both National Parks.

**Data accessibility**

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

**Statements and Declarations**

On behalf of all authors, the corresponding author states that there is no conflict of interest.

**Acknowledgments**

We acknowledge Ariel Cruz Villacorta, Noemí Bocanegra Castillo, Arminda Mejía Rebollo, Claudia Pérez Estrada, and Enrique Morales Bojórquez of CIBNOR for assistance in laboratory work. We thank Erick Treviño Balandra for his assistance in the statistical analyses and the Marine BioGenomics group for support and suggestions in the early stages of preparing the manuscript. This work was funded by the project 10023 of PEP Program of CIBNOR to EFB, grant PROMEP-220265 to APRT, P/PIFI-2010-14MSU0010Z-10 to ACM, NGS-55349R-19 to APRT, PROCER/CCER/DROP/C/09/2016 to ALCM, Marine BioGenomics funding and National Geographic Society.

**References**

46. Witzany G (2014) Biocommunication of animals. Springer Netherlands, Dordrecht

**Figures**
Figure 1

Study sites in the central Mexican Pacific. Black star denotes collection site.
Figure 2

Morphological traits used to detect sexual dimorphism. (a) corallite density (CDe) (b) corallite diameter (CDi), number of adjacent corallites (AC), distance of adjacent corallites (DaC), and corallite area (CAR). Descriptions of each morphological trait are summarized in Table 1.
Figure 3

Sexual dimorphism in morphological and corallite traits of *Porites lobata* corals. Mean values (± standard error) of male (blue) and female (red). Numbers in parenthesis indicate the number of colonies. Significance level: t-test, * p < 0.05.
Figure 4

Canonical discriminant analysis between *Porites lobata* and *Porites panamensis* morphological traits. Each symbol represents one colony. *P. panamensis*: Male (N= 12), Female (N= 19), *P. lobata*: Male (N= 12), Female (N= 6). CDe= corallite density, CDi= corallite diameter, AC= adjacent corallites, and DaC= distance of adjacent corallites.
Figure 5

Modularity differences between sexes of *Porites lobata* (a-b) and of *Porites panamensis* (c-d) Graphical models of the level of modularity of male (a, c) and female (b, d) colonies, where modularity is indicated by the number of trait connections (lines) and their strength (thicker= stronger coordination). (e) SEM image of the traits used in the modularity analysis. CDe= corallite density (yellow), CDi= corallite diameter (blue), DaC= distance of adjacent corallites (brown), and AC= adjacent corallites (purple).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- MSSexualdimorphismESM1.docx
- MSSexualdimorphismESM2Measurements.xlsx